

# Genetic Contributions to Body Weight in Mice: Relationship of Exploratory Behavior to Weight

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## Abstract

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**Objective:** The A/J and C57BL/6J mouse strains differ markedly in their exploratory behavior and their weight gain on a high-fat diet. We examined the genetic contributions of exploratory behavior to body weight and tested for shared, pleiotropic loci influencing energy homeostasis.

**Research Methods and Procedures:** Segregating (A×B6)F2 intercross ( $n = 514$ ) and (B6AF1×A/J)N2 backcross ( $N = 223$ ) populations were studied, phenotyping for weight and exploratory behaviors. Relationships among traits were analyzed by correlations. Weight traits were dissected with a genome-wide scan.

**Results:** Modest correlations were found between exploratory behaviors and weight, explaining 2% to 14% of the variance. Quantitative trait loci (QTL) for body weight at 8 weeks (wgt8), 10 weeks (wgt10), and 2-week weight gain (difference between weeks 8 and 10) on a 6% fat diet were mapped. Two QTL on chromosome 1 (peaks at 66 cM and 100 cM; *Bw8q1*) affected wgt8 [likelihood of the odds ratio (Lod), 3.0 and 4.4] and wgt10 (Lod, 2.2 and 3.4), respectively. In the backcross, a significant QTL on chromosome 4 (peak at 66 cM; *Bw8q2*) affected wgt 8 (Lod, 3.3) and wgt10 (Lod, 3.1). For 2-week weight gain, suggestive QTL were mapped on chromosomes 4 and 6. The chromosome 6 QTL region overlaps a human 7q locus for obesity. A search for between-strain sequence polymorphisms in the leptin and *NPY* genes was unrevealing.

**Discussion:** In mice, loci influencing exploratory activity play a modest role in body-weight regulation. Some forms

of obesity may emerge from loci regulating normal body weight.

**Key words:** body weight, locomotor activity, chromosomal mapping, quantitative trait loci (QTL), individual differences

## Introduction

The development and regulation of body weight is a complex trait with biological, psychological, and environmental influences. From twin studies, the heritability of obesity and fat distribution has been estimated at 30% to 80% (1–3). Although genetic predisposition is important, the regulation of body weight centers on the balance between intake and energy expenditure, which has a strong environmental component (4,5). Single-mutant and transgenic-knockout rodent models have defined many important genes such as *leptin*, *leptin receptor*, *tubby*, and *agouti* [reviewed in Refs. (6–8)], but human mutations in these genes have been uncommon (9,10). The homeostatic regulation of appetite, weight, energy expenditure, and adiposity occurs at numerous hierarchical levels with genetic heterogeneity among individuals. From the perspective of energy regulation, Ravussin (11,12) has examined the total daily-energy expenditure of individuals in a fixed environment and has found considerable variation in spontaneous physical activity. Low levels of physical activity predict an increased risk of obesity in children and adults (13). In Pima Indian children, studies have suggested that plasma leptin concentrations are significantly correlated ( $r = 0.26$ ) with body weight. Leptin has been hypothesized to mediate increased physical activity through activation of the sympathetic nervous system, as well as by decreasing food intake (14). Similarly, fear-like behavior and “stress” may contribute to body-weight regulation through the activation of the central and peripheral sympathetic nervous systems and the hypothalamic-pituitary-adrenal axis (15–17). In small mammals and human infants, adrenergic activation of  $\beta$ -receptors innervating brown adipose tissue increases heat dissipation through the mitochondrial uncoupling protein

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UCP-1. Finally, the role of nonexercise-activity thermogenesis (“fidgetiness”) as a contributor to individual variation in body-weight gain has been highlighted from over-feeding experiments (18).

In rodents, numerous investigators have successfully mapped many quantitative trait loci (QTL)<sup>1</sup> for body-weight regulation, obesity-related traits, and energy balance from F2 intercrosses and backcrosses (9,19–32), although crosses derived from the A/J and C57BL/6J (B6) inbred lines have not been studied. The A/J and B6 strains of mice differ markedly in a number of behavioral traits (see Appendix 1), with the A/J mouse exhibiting significantly inhibited exploratory behavior. Compared with the B6 strain, the A/J mouse differs in the following ways: 1) open field (O-F) behavior involving markedly fewer rearings, less spontaneous activity (ambulation), and more defecations; 2) fear-like behavior involving fewer transitions in the light-dark (L-D) paradigm (33); and 3) stress-induced hyperthermia paradigms involving less temperature elevation (34,35). Likewise, body-weight regulation, leptin levels, and thermogenesis differ markedly for these strains after feeding of a high-fat diet (36–39). Specifically, the obesity and hyperglycemia-prone B6 mice have less diet-induced thermogenesis (lower core body temperature elevation), lower levels of UCP-1 expression in adipose tissue, and lower leptin levels compared with the obesity resistant A/J strain (40,41). We hypothesized the existence of pleiotropic loci (e.g., processing enzymes, transcriptional factors, and/or post-receptor signaling molecules) that regulate both body weight and exploratory behavior and that genetically differ in these strains, acting through the neuro-endocrine system. Genetic crosses were made to test this hypothesis in an assumption-free strategy and to answer the following questions:

1. To what extent do spontaneous activity and fear-like behaviors contribute to body weight?
2. Do the QTL mapped for body weight and behavior (activity or fear-like) co-localize?
3. Do the QTL for body weight gain coincide with obesity-related QTL?

This report describes the genetic dissection of body-weight traits in intercross and backcross populations.

## Research Methods and Procedures

### Animals

Male and female A/J (A), C57BL/6J (B6), B6AF1, and F2 hybrid mice, reared on NIH31M (Zeigler Bros, Inc.,

Gardners, PA) (6% of calories from fat) ad libitum, were obtained from The Jackson Laboratory (Bar Harbor, ME) at 7 to 8 weeks of age. The same cohorts of mice were used for both behavioral and weight-related QTL mapping. (B×A)F2 mice ( $n = 514$ ) were derived from an intercross of B6AF1 mice and housed at NIH. N2 mice ( $n = 223$ ) were produced at University of Texas Southwestern (UTSW) from a backcross of B6AF1 females and A males. All mice were housed in SPF facilities with 4 to 5 animals per cage with food and water ad libitum. The NIH diet was 6% fat NIH31M. The UTSW diet was Teklad 7002 (6% fat; Harlan Teklad, Madison, WI). Animals were maintained under a 12-hour:12-hour L-D cycle with lights on at 6:00 AM. F2 mice were identified by ear notching at 7 to 8 weeks of age, followed by 10 days of adaptation before phenotyping. N2 mice were ear notched at 4 weeks of age and tested at 7 to 8 weeks of age. Mice were individually weighed to the nearest 0.1g at 8 and 10 weeks of age. Mice were subjected to sequential testing in behavioral paradigms: exploratory behavior in an O-F and L-D paradigm. For the backcross offspring, L-D transition testing was not performed. All experiments followed the NIH Guide for the Care and Use of Laboratory Animals and received institutional Animal Care and Use approval.

### DNA Preparation and Genotyping

DNA was prepared and genotyped as described previously (42). Briefly, individual mouse DNA was genotyped by polymerase chain reaction (PCR) with MapPair primers (Research Genetics/Invitrogen, Carlsbad, CA). A panel of 102 approximately equally-spaced CA repeat microsatellite DNA markers were selected from the integrated Whitehead Institute Genome Center Web site (<http://www-genome.wi.mit.edu/>) and the Mouse Genome Database (MGD) (<http://www.informatics.jax.org/>), providing a whole genome screen at a spacing of ~14 cM.

### Exploratory Behavior in an O-F and L-D Transition

The procedures and results of these behavioral studies: exploratory behavior in an O-F and the L-D transitions have been previously described in detail (42–44). In the O-F test, an animal is placed in a novel, brightly lit, open arena. Traditionally, the O-F test has been considered to be a mouse model of “temperament” or “emotionality” [reviewed in Refs. (45,46)]. Three behavioral parameters were measured; namely, the distance traveled (ambulation), vertical movements (rearing), and the tendency to move away from the central zone (“thigmotaxis”). Thigmotaxis indicates the natural tendency of mice to seek a wall (“wall hugging”). The related variable “center time” is measured as the amount of time that a mouse spends away from the wall during O-F testing. Some investigators have viewed O-F behavior as a form of “exploratory” behavior, whereas others have suggested that it reflects escape behavior, a

<sup>1</sup> Nonstandard abbreviations: QTL, quantitative trait locus (loci); O-F, open field; L-D, light-dark; UTSW, University of Texas Southwestern; PCR, polymerase chain reaction; MGD, Mouse Genome Database; Tde1, total distance ambulated epoch 1; VM15, vertical movements during the first trial of 15 minutes; Lod, likelihood of the odds ratio; wgt8, 8-week body weight; wgt10, 10-week body weight; AvgCtrT, average center time.

central state of arousal, fear, or “a nonspecific excitability level.” The L-D test is a naturalistic conflict test, where an animal is initially placed in the bright side of a two-compartment box. There is a conflict between the natural drive to explore vs. the avoidance of bright light. The number of transitions between the light and dark zones is a measure of fear-like behavior, and anxiolytics increase the number of transitions in a dose-dependent fashion (33,47–49). Briefly, mice were tested for O-F measures during two trials, with an inter-trial interval of 14 days. Only the initial tests at 8 weeks were utilized, preventing “carry-over” effects. Each trial consisted of three consecutive 5-minute epochs (e1, e2, e3) (15 minutes) in an animal-activity monitor (Digiscan RXYZ8; Omnitech Electronics, Inc., Columbus, OH; or Opto-Varimex-3, Columbus Instruments, Columbus, OH; 730 lux at cage floor). The square field had dimensions of  $43 \times 43 \times 30.5$  cm, and the vertical sensors were positioned 8 cm above the cage floor. The following dependent measures were assessed: 1) initial total distance (TDe1), the distance traveled or ambulated during the initial 5-minute epoch, epoch 1; 2) vertical movements (VM15), the sum of vertical movements (rearings) during the first trial of 15 minutes; and 3) average center time (AvgCtrT), the time (calculated in seconds) spent by the animal  $>1$  cm from the cage walls averaged during the second and third 5-minute epochs [(center time epoch 2 + center time epoch 3)/2]. One day after O-F testing, L-D testing was performed in a single 10-minute session, and the number of L-D transitions was the dependent variable.

**Statistical Analysis.** Weight gain was measured as a simple difference score, subtracting the body weight at 8 weeks (wgt8) from the body weight at 10 weeks (wgt10). Body weight and weight gain measures were assumed to be normally distributed and treated as continuous variables. Non-normally distributed behavioral traits from O-F and L-D testing were square-root transformed as described previously (42,43). Overall, differences in body weight were compared across sex, strain, and generation by one-way and two-way ANOVA (Statview 5.1 and SuperAnova, Abacus Concepts and JMP 3.02; SAS Institute, Cary, NC). Significant genotype-by-gender interactions were detected by two-way ANOVA. Significance testing utilized post hoc tests in one-way ANOVA and planned comparisons of means for two-way ANOVA. The overall strategy for linkage involved a three-step approach. First, selected mice from the high and low 9% phenotypic extremes of the weight traits' distribution were tested by  $\chi^2$  tests of independence between markers and trait. Then, for linkage estimation and QTL localization, we utilized genotypic and phenotypic data from the whole population with Mapmaker/QTL (50–52) and Map Manager QTXb14 (53) programs to estimate the percentage of the phenotypic variance explained for each QTL, using sex in the background as a regressor. For interpreting these linkage results, we used the

published guidelines (54). For the F2 population, the thresholds for suggestive and significant linkage were likelihood of the odds ratio (Lod) scores of 2.8 and 4.3, respectively. For the N2 population, the threshold values for suggestive and significant linkage were Lod scores of 1.9 and 3.3, respectively. Finally, the data were modeled by stepwise linear regression and interval mapping to find the best-fitting model, incorporating sex effects and joint QTL effects (55,56). Epistatic interactions were examined by three-way ANOVA, testing for interactions among mapped loci with sex as the third factor and by linear regression analysis and testing for statistical interactions among mapped loci as described (56,57).

**Sequence Comparison and Examination of Candidate Genes.** Leptin sequences from A/J and B6 strains were determined on both strands using automated DNA sequencing using genomic DNA for the 5' promoter (0 to  $-300$ , where the transcriptional initiation site is located at  $+1$ ), and for the coding determining regions using reverse-transcriptase PCR with PCR primers as described (58–60). The Celera Mouse RefSNP database (<http://www.celeradiscovery.com>) was used to examine each QTL region for polymorphisms between A/J and C57BL6/J, as well as specific candidate genes, reporting only those single-nucleotide polymorphisms covered at least two or more times per strain.

## Results

The body-weight phenotypic data for the parental lines, the F1, the F2, and the N2 populations are shown in Table 1. Among the parental, F1, and F2 groups for the dependent variables of wgt8, wgt10, and 2-week weight gains, there were significant sex differences for each population [ $F(1,343) = 187, p < 0.0001$  at wgt8;  $F(1,343) = 141, p < 0.0001$  at wgt10;  $F(1,343) = 8.1, p < 0.0048$  for 2-week weight gain]. Similarly, for the N2 cross, sex differences (M>F; weight gain was greater among males) were highly significant for all the dependent variables [wgt8:  $F(1217) = 142, p < 0.0001$ ; wgt10:  $F(1,221) = 167, p < 0.0001$ ; 2-week weight gain:  $F(1,217) = 7.1, p < 0.0080$ ; note: for 2-week weight gain, B6 (M>F) and A/J (F>M)]. Although the means of the parental strains differed significantly for all body-mass variables, the magnitude of these differences (effect sizes) ranged from 0.32 for females at wgt8 to 0.86 for females at 2-week weight gain. For comparison, the effect sizes of 8.5 and 4.7 were found for differences in these parental strains for O-F exploratory total distance (TDe1) traveled and L-D transition behavior, respectively.

Tables 2 and 3 show the correlations and relationships among the dependent variables measured in the segregating populations. As expected, the wgt8 measures were highly correlated with wgt10 ( $r \geq 0.73, p < 0.0001$ ). In the F2 generation, 2-week weight gain was moderately correlated with wgt8 and wgt10. In the backcross, 2-week weight gain

**Table 1.** Phenotypic means of the Parental lines, F1, F2, and N2 mice

Origin	Sex	Weight (grams)								
		<i>N</i>	8 weeks	SD	<i>N</i>	10 weeks	SD	<i>N</i>	2-Week weight gain	SD
A/J	M	14	25.3	2.7	14	26.9	2.4	14	1.6	0.6
	F	15	19.6	2.1	15	21.9	2.2	15	2.3	1.3
B6	M	15	26.4	1.6	15	28.5	2.4	15	2.1	1.1
	F	15	19.0	1.8	15	20.2	1.9	15	1.2	1.0
(B×A)F1	M	10	25.7	2.7	10	28.2	3.8	10	2.5	3.2
	F	10	21.3	1.2	10	22.1	1.7	10	0.9	1.3
F2	M	274	26.8	2.7	149	32.2	3.6	149	4.5	1.7
	F	242	21.2	2.4	123	24.5	3.2	123	2.9	1.8
N2	M	118	20.7	2.3	120	22.5	2.3	118	1.8	1.3
	F	101	17.3	1.9	103	18.6	2.1	101	1.4	1.5

was negatively correlated with wgt8, but positively correlated with wgt10. The correlations among the different measures of exploratory behavior (TDe1, VM15, and AvgCtrT) and L-D behavior demonstrate that these measures are related components of exploration and fear-like behavior. In general, the correlations among spontaneous exploratory activity, fear-like behavior, and weight revealed a weak relationship between these behavioral variables and body mass. The most significant correlations were in the F2 intercross females, in which O-F total distance epoch 1 (TDe1) and L-D behavior (L-D) explained 4.4% to 13.7% of the variance in weight traits. The negative correlations between behavioral measures and weight traits agree with the expectation that the more active mice in exploratory behavior and L-D behavior have the lower weight and weight gain.

A whole-genome scan was performed on the overall segregating populations, and the QTL mapping results are

shown in Table 4, along with previously mapped QTL in these regions. In the F2 cross, QTL mapping revealed two loci on chromosome 1, affecting both wgt8 and wgt10. The one Lod interval of the significant distal chromosome 1 locus (77 to 102 cM; *Bw8q1*) encompassed a previously mapped region with confirmed QTL for exploratory behavioral traits (TDe1 and VM15) (42,61–63). The F2 mice inheriting the *B/B* genotype at *Bw8q1* locus were more active and weighed less than the *A/A* QTL. Using the mapped loci as regressors, thereby partitioning out the variance attributable to already-mapped loci, no additional QTL were mapped upon rerunning the mapping analyses. Neither sex-by-genotype interactions nor epistatic interactions between loci were detected for these loci.

The backcross mapped a locus on distal chromosome 4, *Bw8q2*, affecting wgt8 significantly and wgt10 suggestively, explaining 4% and 3% of the variance, respectively (Table 5). *Bw8q2* had a significant sex-specific effect. In

**Table 2.** Pearson's correlation coefficients among measures in the F2 mice

Variable	wgt8	wgt10	2-Week weight gain	TDe1	VM15	L-D	AvgCtrT
wgt8	1.00	0.91	0.34	<b>-0.37</b>	-0.07	-0.16	-0.10
wgt10	0.89	1.00	0.69	<b>-0.37</b>	-0.13	<b>-0.24</b>	-0.09
2-Week weight gain	0.26	0.67	1.00	<b>-0.21</b>	-0.17	<b>-0.27</b>	-0.02
TDe1	-0.08	-0.13	-0.16	1.00	0.59	0.45	0.36
VM15	-0.11	-0.17	-0.18	0.51	1.00	0.41	0.18
L-D	<b>-0.21</b>	<b>-0.21</b>	-0.10	0.34	0.46	1.00	0.16
AvgCtrT	-0.14	-0.14	-0.07	0.37	0.08	0.09	1.00

Below the diagonal (values of 1.00) males (*N* = 123); above the diagonal females (*N* = 143). Boldface values are the relevant and significant correlations (*p* < 0.001).

**Table 3.** Correlation coefficients among measures in the N2 mice

Variable	wgt8	wgt10	2-Week weight gain	TDe1	VM15	AvgCtrT
wgt8	1.00	0.733	-0.27	<b>0.34</b>	-0.01	0.10
wgt10	0.85	1.00	0.46	<b>0.21</b>	-0.02	0.00
2-Week weight gain	-0.22	0.33	1.00	-0.15	-0.02	-0.13
TDe1	<b>0.22</b>	<b>0.18</b>	-0.06	1.00	0.25	0.37
VM15	0.09	0.03	-0.11	0.52	1.00	0.03
AvgCtrT	<b>0.26</b>	<b>0.19</b>	-0.11	0.41	0.25	1.00

Below the diagonal (values of 1.00) males ( $N = 118$ ); above the diagonal females ( $N = 97$ ). Boldface values are the relevant and significant correlations ( $p < 0.001$ ).

males, mean weights for the *A/B* vs. *A/A* allele were 21.3 g vs. 19.6 g at wgt8 and 23.0 g vs. 21.7 g at wgt10, respectively. Females showed no significant differences. The prominent candidate genes in this region include a serotonin receptor cluster (*Htr6*, *Htr1D*), 3-hydroxy-3-methylglutaryl-coenzyme A lyase (*Hmgc*), and syndecan 3. Suggestive loci on chromosomes 8, 14, and 17 were mapped. The chromosome 17 locus encompasses the candidate genes Tubby-like protein 4 (*Tulp4*) and insulin-like growth factor 2 receptor (*Igfr2*). For weight gain, suggestive loci on proximal chromosomes 4 and 6 were mapped. The proximal chromosome 4 weight-gain locus affected only females. The suggestive chromosome 6 QTL region for weight gain encompasses several candidate genes, including leptin and *NPY*. A comparison of the *A/J* and *B6* strains' leptin sequences in the 5' promoter region (300 base pairs) and the coding-determining sequence revealed identical sequences (data not shown). A Celera database search confirmed the absence of sequence polymorphisms between strains in these gene regions. Similarly, a search (accessed February 2003) for specific candidate genes and for genes with polymorphisms in these regions was unrevealing, with some exceptions. The *5Htr1D* locus had a missense C/T mutation changing the *B6* [Val(GTG)22 to Ala(GCG)22] in the *A* strain (Celera single-nucleotide polymorphism identification no. mCV22559909). Putative nonsense mutations in the *A* strain were found in the *Bwq1* region for the 40S ribosomal protein S11 [mCV23151540; Arg(CGA)233Stop(TGA)] and in the *Bw8q2* region for the ribosomal protein L18 [mCV23151540; Gln(CAA)-539Stop(TAA)] plus the loss of an acceptor splice site for the ribosomal protein 23A (mCV23411689). Several other putative nonsense mutations were noted in novel genes.

### Discussion

We have examined the relationship between normal body weight and exploratory behavior in mice in order to test hypotheses about the role of physical activity and "stress" in

contributing to energy balance and weight regulation (15,64). Initially, studies of Pima Indian children found that total energy expenditure and the level of physical activity correlated with plasma leptin concentrations, independently of percentage of body fat (14). The investigators hypothesized that leptin may mediate increased physical activity through activation of the sympathetic nervous system, as well as by decreasing food intake. Subsequent studies have demonstrated spontaneous activity as a risk factor for obesity, and overfeeding studies have suggested that nonexercise-activity thermogenesis plays a critical role in weight gain (18), explaining inter-individual variation in weight gain. We examined the association of exploratory behavior and normal weight gain using crosses between strains differing in their exploratory behavior. The most important finding of this study is that exploratory activity explained a modest 2% to 14% of the variance in weight traits under these conditions. This result supports the findings of previous overfeeding experiments in the obesity-prone *B6* and obesity-resistant *A/J* mice, which found that spontaneous motor activity did not seem to be a major factor contributing to weight gain (37). Similarly, in overweight undergraduate students, no differences in activity were noted using actometers, but differences have been found in morbidly obese individuals (65).

Previous work has extensively analyzed the genetics of obesity and body weight-related traits in rodents and human studies [reviewed in Refs. (4,7,9,66,67)]. The body weights shown in Table 1 were similar to those of mice in the Mouse Phenome project fed on a 11% fat diet. In general, we confirm the generalization by Cheverud et al. (29) that body weight results from "many genes with relatively small effect." These crosses detected only the largest of these small effects. The second most important findings are the mapping of statistically significant QTL near D1Mit116 (Lod score, 4.4; 100 cM; *Bw8q1*) and D4Mit68 (Lod score, 3.3; 66cM; *Bw8q2*) and the confirmation of several other loci. The QTL mapped in this study span an interval of roughly

**Table 4.** F2 QTL mapping summary

Trait	Nearest marker	Peak position (cM)	1 Lod (confidence interval)	Peak Lod score	Variation %	Additive	Dominance	ANOVA results				QTL name	QTL in interval		
								<i>F</i> ( <i>df</i> )	<i>p</i> value	Sex	A/A			A/B	B/B
wgt8	DIMit116	100	95 to 111	<b>4.4</b>	<b>2</b>	-0.72	0.54	8.3 (2,490)	0.003	M	27.2	26.8	26.1	<b>Bw8q1</b>	<i>Wgt3q2</i> , <i>Wgt6q2</i> <i>Emo1</i> , <i>Exq3</i> <i>Bwtq1</i> , <i>Bgeq1</i> , <i>Fattq</i> , <i>IBw17</i> , <i>Wt3q1</i> , <i>Obq8</i> , <i>Emo3</i> , <i>Exq2</i>
	DIMit191	66	44 to 73	3.0	1	-0.44	0.82	6.1 (2,450)	0.0025	M	27.0	27.1	26.5		
wgt10	DIMit116	100	95 to 105	3.4	3	-1.12	1.18	6.9 (2,263)	0.0012	M	32.8	32.6	30.9		
	DIMit191	66	43 to 73	2.2	2	-0.52	1.49	4.5 (2,260)	0.0122	M	32.1	33.2	31.2		
										F	25.4	26.1	24.5		

Peak position is the most likely QTL position by interval mapping, given as distances from the centromere based on MGD locations of markers. The 1 Lod support intervals provide an estimated range for the QTL location. Significant loci are bolded and named. For the F2 population, the threshold values for suggestive and significant linkage were Lod scores of 2.8 and 4.3, respectively. These suggestive and significant levels of linkage correspond to genome-wide error rates, i.e., that such results would occur one time at random in a genome scan and 0.05 times in a genome scan, respectively. The QTL additive and dominance effects were estimated with Map Manager QTXb14. At each locus, mice in the segregating populations have their alleles represented by "A" and "B" for derivation from A/J mice and B6/J mice, respectively. ANOVA permits classification of F2 with respect to the nearest marker locus to detect single marker significance, comparing the phenotypic means of genotypic subpopulations (A/A, A/B, and B/B). Overlapping QTL lists the abbreviations of previously mapped QTL in the interval as referenced by the MGD. Boldface numerical values are for loci with significant linkage.

**Table 5.** N2 QTL mapping summary

ANOVA results												
QTL mapping results					Locus × sex							
Trait	Peak position (cM)	1 Lod interval	Peak Lod score	% Variation	Additive	interaction, F (df)	p value	Sex	Locus main effect, F (df)	QTL name		
Wgt8	D4Mit68	54 to 75	3.3	4	1.08	4.6 (1,209)	0.0335	M	19.6 21.3	17.33 0.0001	<b>Bw8q2</b> , <b>Bw7</b> , <b>Afpq2</b>	
	D8Mit4	0 to 40	2.3	2	-0.91	0.1 (1,210)	NS	F	17.1 17.5	0.97	NS	<b>Afw2</b> , <b>Aaiq1</b> , <b>Aaiq2</b>
	D14Mit75	48 to 69	2.2	2	0.89	0.1 (1,212)	NS	M	21.1 20.3	7.90	0.0054	<b>Adip4</b> , <b>BgeQTL7</b> , <b>Wg3</b> , <b>Bwq3</b>
Wgt10	D4Mit68	64 to 84	3.1	3	1.2	1.0 (1,213)	NS	M	21.7 23.0	11.8	0.0007	
	D14Mit75	48 to 69	2.6	3	1.04	0.8 (1,216)	NS	M	21.8 23.0	11.9	0.0007	
	D17Mit46	0 to 23	2.3	2	0.95	0.2 (1,211)	NS	M	22.0 23.0	9.1	0.0029	<b>Obq4</b>
2-Week weight gain	D4Mit53	5 to 28	2.9	6	1.5	3.8 (1,210)	0.0538	M	1.82 1.86	0.02	NS	<b>Bwq4</b> , <b>Bglq4</b>
	D6Mit74	8 to 39	2.5	5	-1.36	0.3 (1,204)	NS	M	2.02 1.70	7.95	0.0053	<b>Triglq1</b>
								F	1.65 1.14	4.9	0.0285	<b>Obql3</b> , <b>Bw18</b>

Peak position is the most likely QTL position by interval mapping, given as distances from the centromere based on MGD locations of markers. The 1 Lod support intervals provide the estimated range for location of the QTL. Significant loci are bolded and named. For the N2 population, the threshold values for suggestive and significant linkage were Lod scores of 1.9 and 3.3, respectively. These suggestive and significant levels of linkage correspond to genome-wide error rates, i.e., that such results would occur one time at random in a genome scan and 0.05 times in a genome scan, respectively. The QTL additive effects were estimated with Map Manager QTXb14. Two-way ANOVAs (factors = marker locus and sex; dependent variable = trait) examined locus × sex interactions. For nonsignificant sex-by-genotype interactions, samples were pooled across sex, and overall F values are reported. For significant sex-by-genotype interactions, separate ANOVA analyses were reported split by sex. One-way ANOVAs classifies N2 mice with respect to the nearest marker locus to detect single-point marker significance, comparing the phenotypic means of genotypic subpopulations (A/B vs. A/A). Abbreviations of the QTL overlapping the identified regions are listed as referenced by MGD. NS, not significant. Boldface numerical values are for loci with significant linkage.

15 to 20 cM, encompassing 450 to 1000 genes, and overlap with previously reported QTL as shown in Table 4. The selection of likely candidate genes is precarious. A Celera database search for polymorphic genes in these regions reduced the list of candidate genes. Many of these genes were novel with unknown function. No obvious gene(s) has emerged from this search to date (accessed February 2003; data not shown). The locus on distal chromosome 1 for body-weight regulation overlaps previously mapped O-F activity loci (42–44,61,63,68). Although interesting, the coincidence of localization should not be over-interpreted. The possibility of multiple QTL in this peak is quite likely for the O-F traits. However, the intriguing possibility of pleiotropic regulatory molecules such as regulator of G-signaling proteins in the chromosome 1 region remains for further study. Fine mapping will clarify this issue, and positional cloning of these loci will ultimately demonstrate whether a pleiotropic locus exists.

Because the obesity-prone B6 mouse has markedly lower leptin levels compared with the obesity-resistant A/J mouse after 1 month of high-fat feeding (38,41), our mapping of a chromosome 6 locus controlling body-weight gain near the leptin and *NPY* loci was of potential interest. Second, the human syntenic region on 7q was identified in two large genome-wide scans for BMI, namely the National Heart, Lung, and Blood Institute Family Heart Study (7q32.3; Lod score, 4.9) (69) and in the HyperGEN black cohort (7q22.3; Lod score, 2.36) of the National Heart, Lung, and Blood Institute Blood Pressure Program (70). Because these weight-gain QTL overlap QTL for obesity-related traits, perhaps some forms of obesity result from the same loci regulating normal weight gain. In addition, effects of relatively rare mutations in humans have supported leptin's key role in body weight (10,71). Therefore, leptin and *NPY* were prioritized as plausible candidate genes. The lack of polymorphic sequences in the 5' promoter sequence and coding determining sequences of leptin, however, excludes these regions of DNA. Similarly, the *NPY* coding sequence demonstrated no polymorphisms. We cannot rule out polymorphisms in surrounding transcriptional regulatory elements. Of note, transcriptional regulatory elements have been found at a distance of over 270 kilobase pairs from the promoter (72). The finding of mutations in the S11, L23A, and L18 ribosomal protein genes is intriguing. In *Drosophila*, mutations in ribosomal proteins cause the haplo-insufficient *Minute* phenotype with reduced growth and reduced body size (73). However, in mice and human genomes, these ribosomal genes are present in multiple redundant copies. Nevertheless, whether the reduction in quantity of these ribosomal proteins may contribute to body-weight traits is a testable hypothesis.

Some limitations of this study should be noted. First, the QTL mapped have relatively small effect sizes. Second, because prior genetic studies have demonstrated that ab-

dominal-fat and muscle weights are highly correlated ( $r > 0.61$ ) with body weight, body weight alone was used as the dependent measure. Limited power and small QTL effect sizes resulted from the selection of parental strains with modest differences in body weight, sex effects, and limitations in sample size. Although this strain combination provides the benefits of well-defined differences in obesity-related traits and rich genomic resources for gene discovery, these crosses offer limited power for QTL detection of weight with lower fat diets. Third, this study was performed over several years at two different physical locations (NIH for the F2 and UTSW for the N2). Hence, although housing density, breeding, and investigators' procedures were identical, the F2 and N2 populations were reared with subtle environmental differences and different commercial sources of their low-fat 6% diets. Fourth, although we were attentive to handling mice gently, we cannot formally rule out the unlikely possibility of a genotype-by-"stress" (behavioral testing) interaction, leading to some minimal differences in body weight at wgt10 and at 2-week weight gain. In the parental strains and the F1 hybrids, no such genotype-by-stress interaction has been detected for body weight (data not shown). Fifth, the general problem of adjusting for differences in initial, baseline values is termed Lord's paradox. Given initial differences in baseline weight, there is no consensus on a method for correcting the weight-gain trait values over 2 weeks. We have selected a simple difference score to measure weight gain over a 2-week period, whereas others might prefer alternative approaches [reviewed in Ref. (74)]. Finally, we have not experimentally verified the sequence polymorphisms found in the Celera database.

Given that mice eat approximately 15% to 20% of their weight daily, differences between mouse and human regulation of energy expenditure might be anticipated. In contrast to humans, spontaneous exploratory behavior in mice seems to explain only a very modest percentage of body-weight and weight-gain variance. Hence, this fact may partially explain the apparent paradox of the strain difference in response to a high-fat diet, whereby the less active A/J strain is obesity-resistant and the high-activity B strain is obesity-prone. As expected, increased exploratory behavior does indeed decrease body weight with most QTL in the F2 cross derived from the B strain that decreases body weight. However, consistent with the literature suggesting "many small effects," these loci each explain only 1% to 3% of the phenotypic variance. The QTL for normal weight mapped in this study overlap QTL for obesity-related traits (75), suggesting that predisposing loci for normal weight gain may also lead to obesity. The differential regulation between B6 and A/J mice of leptin and *UCP1* gene expression, leptin sensitivity, adrenergic responsivity, and thermogenic activity warrants future exploration (40,41,76). In response to acute stress and its attendant sympathetic acti-

vation, A/J mice have a small temperature elevation compared with the B6 (34,35). Yet, after 2 weeks of a high-fat diet, the A/J mice develop a higher core body temperature compared with the B6 (38). This discrepancy in thermogenic regulation hints at differences in post-receptor signaling, which suggests the need for further searches for pleiotropic molecules, such as regulator of G-signaling proteins, adipokines, transcription factors, and neuropeptide processing enzymes. Finally, defining the precise molecules underlying these QTL and improving our understanding of the early development of diet-induced obesity in the A/J vs. B6 strains may lead to a greater mechanistic understanding of the biological determinants of weight regulation.

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### References

1. **Sorensen TI.** The genetics of obesity. *Metab Clin Exp.* 1995; 44:4–6.
2. **West DB.** Genetics of obesity in humans and animal models. *Endocrinol Metab Clin North Am.* 1996;25:801–13.
3. **Bouchard C.** Genetic determinants of regional fat distribution. *Hum Reprod.* 1997;12:1–5.
4. **Bray G, Bouchard C, Jansen WP, eds.** *Handbook of Obesity.* New York: Marcel Dekker, Inc.; 1998.
5. **Spiegelman BM, Flier JS.** Obesity and the regulation of energy balance. *Cell.* 2001;104:531–43.
6. **Barsh GS, Farooqi IS, O'Rahilly S.** Genetics of body-weight regulation. *Nature.* 2000;404:644–51.
7. **Robinson SW, Dinulescu DM, Cone RD.** Genetic models of obesity and energy balance in the mouse. *Annu Rev Genet.* 2000;34:687–745.
8. **Leibel RL, Chung WK, Chua SC Jr.** The molecular genetics of rodent single gene obesities. *J Biol Chem.* 1997;272:31937–40.
9. **Rankinen T, Perusse L, Weisnagel SJ, Snyder EE, Chagnon YC, Bouchard C.** The human obesity gene map: the 2001 update. *Obes Res.* 2002;10:196–243.
10. **Farooqi IS, Keogh JM, Kamath S, et al.** Partial leptin deficiency and human adiposity. *Nature.* 2001;414:34–5.
11. **Ravussin E.** Metabolic differences and the development of obesity. *Metab Clin Exp.* 1995;44:12–4.
12. **Ravussin E.** Low resting metabolic rate as a risk factor for weight gain: role of the sympathetic nervous system. *Int J Obes Relat Metab Disord.* 1995;19:S8–9.
13. **Filozof C, Gonzalez C.** Predictors of weight gain: the biological-behavioural debate. *Obes Rev.* 2000;1:21–6.
14. **Salbe AD, Nicolson M, Ravussin E.** Total energy expenditure and the level of physical activity correlate with plasma leptin concentrations in five-year-old children. *J Clin Invest.* 1997;99:592–5.
15. **Björntorp P.** Do stress reactions cause abdominal obesity and comorbidities? *Obes Rev.* 2001;2:73–86.
16. **Dulloo AG.** A sympathetic defense against obesity. *Science.* 2002;297:780–1.
17. **Rayner DV, Trayhurn P.** Regulation of leptin production: sympathetic nervous system interactions. *J Mol Med.* 2001; 79:8–20.
18. **Levine JA, Eberhardt NL, Jensen MD.** Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science.* 1999;283:212–4.
19. **Suto J, Matsuura S, Imamura K, Yamanaka H, Sekikawa K.** Genetics of obesity in KK mouse and effects of A(y) allele on quantitative regulation. *Mamm Genome.* 1998;9:506–10.
20. **Morris KH, Ishikawa A, Keightley PD.** Quantitative trait loci for growth traits in C57BL/6J × DBA/2J mice. *Mamm Genome.* 1999;10:225–8.
21. **Moody DE, Pomp D, Nielsen MK, Van Vleck LD.** Identification of quantitative trait loci influencing traits related to energy balance in selection and inbred lines of mice. *Genetics.* 1999;152:699–711.
22. **York B, Lei K, West DB.** Inherited non-autosomal effects on body fat in F2 mice derived from an AKR/J × SWR/J cross. *Mamm Genome.* 1997;8:726–30.
23. **Brockmann GA, Haley CS, Renne U, Knott SA, Schwerin M.** Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth. *Genetics.* 1998;150:369–81.
24. **Rance KA, Hill WG, Keightley PD.** Mapping quantitative trait loci for body weight on the X chromosome in mice. I. Analysis of a reciprocal F2 population. *Genet Res.* 1997;70: 117–24.
25. **Rance KA, Heath SC, Keightley PD.** Mapping quantitative trait loci for body weight on the X chromosome in mice. II. Analysis of congenic backcrosses. *Genet Res.* 1997;70:125–33.
26. **Kovacs P, Kloting I.** Mapping of quantitative trait loci for body weight on chromosomes 1 and 4 in the rat. *Biochem Mol Biol Int.* 1998;44:399–405.
27. **West DB, Goudey-Lefevre J, York B, Truett GE.** Dietary obesity linked to genetic loci on chromosomes 9 and 15 in a polygenic mouse model. *J Clin Invest.* 1994;94:1410–6.
28. **Taylor BA, Phillips SJ.** Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. *Genomics.* 1996;34:389–98.
29. **Cheverud JM, Routman EJ, Duarte FA, van Swinderen B, Cothran K, Perel C.** Quantitative trait loci for murine growth. *Genetics.* 1996;142:1305–19.
30. **Keightley PD, Hardge T, May L, Bulfield G.** A genetic map of quantitative trait loci for body weight in the mouse. *Genetics.* 1996;142:227–35.
31. **West DB, Waguespack J, York B, Goudey-Lefevre J, Price RA.** Genetics of dietary obesity in AKR/J × SWR/J mice:

- segregation of the trait and identification of a linked locus on chromosome 4. *Mamm Genome*. 1994;5:546–52.
32. **Warden CH, Fisler JS, Shoemaker SM, et al.** Identification of four chromosomal loci determining obesity in a multifactorial mouse model. *J Clin Invest*. 1995;95:1545–52.
  33. **Mathis C, Paul SM, Crawley JN.** Characterization of benzodiazepine-sensitive behaviors in the A/J and C57BL/6J inbred strains of mice. *Behav Genet*. 1994;24:171–80.
  34. **Liu X, Peprah D, Gershenfeld HK.** Tail-suspension induced hyperthermia: a new measure of stress reactivity. *J Psychiatr Res*. 2003;37:249–59.
  35. **Bouwknicht JA, Paylor R.** Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. *Behav Brain Res*. 2002;136:489–501.
  36. **Surwit RS, Feinglos MN, Rodin J, et al.** Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism*. 1995;44:645–51.
  37. **Brownlow BS, Petro A, Feinglos MN, Surwit RS.** The role of motor activity in diet-induced obesity in C57BL/6J mice. *Physiol Behav*. 1996;60:37–41.
  38. **Surwit RS, Petro AE, Parekh P, Collins S.** Low plasma leptin in response to dietary fat in diabetes- and obesity-prone mice. *Diabetes*. 1997;46:1516–20.
  39. **West DB, Boozer CN, Moody DL, Atkinson RL.** Dietary obesity in nine inbred mouse strains. *Am J Physiol*. 1992;262:R1025–32.
  40. **Surwit RS, Wang S, Petro AE, et al.** Diet-induced changes in uncoupling proteins in obesity-prone and obesity-resistant strains of mice. *Proc Natl Acad Sci U S A*. 1998;95:4061–5.
  41. **Watson PM, Commins SP, Beiler RJ, Hatcher HC, Gettys TW.** Differential regulation of leptin expression and function in A/J vs. C57BL/6J mice during diet-induced obesity. *Am J Physiol Endocrinol Metab*. 2000;279:E356–65.
  42. **Gershenfeld HK, Neumann PE, Mathis C, Crawley JN, Li X, Paul SM.** Mapping quantitative trait loci for open-field behavior in mice. *Behav Genet*. 1997;27:201–10.
  43. **Gershenfeld HK, Paul SM.** Mapping quantitative trait loci for fear-like behaviors in mice. *Genomics*. 1997;46:1–8.
  44. **Gershenfeld HK, Neumann PE, Li X, St Jean PL, Paul SM.** Mapping quantitative trait loci for seizure response to a GABAA receptor inverse agonist in mice. *J Neurosci*. 1999;19:3731–8.
  45. **Archer J.** Tests for emotionality in rats and mice: a review. *Animal Behav*. 1973;21:205–235.
  46. **Walsh RN and Cummins RA.** The open-field test: a critical review. *Psychol Bull*. 1976;83:482–504.
  47. **Crawley J, Goodwin FK.** Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav*. 1980;13:167–70.
  48. **Crawley JN, Davis LG.** Baseline exploratory activity predicts anxiolytic responsiveness to diazepam in five mouse strains. *Brain Res Bull*. 1982;8:609–12.
  49. **Crawley JN.** Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. *Pharmacol Biochem Behav*. 1981;15:695–9.
  50. **Lincoln S, Lander ES.** Systematic detection of errors in genetic linkage data. *Genomics*. 1992;14:604–10.
  51. **Lander ES, Green P, Abrahamson J, et al.** MAPMAKER: an interactive package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*. 1987;1:174–81.
  52. **Paterson AH, Damon S, Hewitt JD, et al.** Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. *Genetics*. 1991;127:181–97.
  53. **Manly KF, Olson JM.** Overview of QTL mapping software and introduction to map manager QT. *Mamm Genome*. 1999;10:327–34.
  54. **Lander E, Kruglyak L.** Genetic dissection of complex traits—guidelines for interpreting and reporting linkage results. *Nat Genet*. 1995;11:241–7.
  55. **Sokal RR, Rohlf FJ.** *Biometry, 3rd ed.* New York: W. H. Freeman; 1995.
  56. **Liu BH.** *Statistical Genomics: Linkage, Mapping, and QTL Analysis.* Boca Raton, FL: CRC Press; 1998.
  57. **Cheverud JM, Routman EJ.** Epistasis and its contribution to genetic variance components. *Genetics*. 1995;139:1455–61.
  58. **He Y, Chen H, Quon MJ, Reitman M.** The mouse obese gene. Genomic organization, promoter activity, and activation by CCAAT/enhancer-binding protein alpha. *J Biol Chem*. 1995;270:28887–91.
  59. **Mason MM, He Y, Chen H, Quon MJ, Reitman M.** Regulation of leptin promoter function by Sp1, C/EBP, and a novel factor. *Endocrinology*. 1998;139:1013–22.
  60. **Hwang CS, Mandrup S, MacDougald OA, Geiman DE, Lane MD.** Transcriptional activation of the mouse obese (ob) gene by CCAAT/enhancer binding protein alpha. *Proc Natl Acad Sci U S A*. 1996;93:873–7.
  61. **Flint J, Corley R, DeFries JC, et al.** A simple genetic basis for a complex psychological trait in laboratory mice. *Science*. 1995;269:1432–5.
  62. **Koyner J, Demarest K, McCaughran J Jr., Cipp L, Hitzemann R.** Identification and time dependence of quantitative trait loci for basal locomotor activity in the BXD recombinant inbred series and a B6D2 F2 intercross. *Behav Genet*. 2000;30:159–70.
  63. **Mott R, Talbot CJ, Turri MG, Collins AC, Flint J.** From the cover: a method for fine mapping quantitative trait loci in outbred animal stocks. *Proc Natl Acad Sci U S A*. 2000;97:12649–54.
  64. **Thorburn AW, Proietto J.** Biological determinants of spontaneous physical activity. *Obes Rev*. 2000;1:87–94.
  65. **Tryon WW.** Activity as a function of body weight. *Am J Clin Nutr*. 1987;46:451–5.
  66. **Comuzzie AG, Williams JT, Martin LJ, Blangero J.** Searching for genes underlying normal variation in human adiposity. *J Mol Med*. 2001;79:57–70.
  67. **Woods SC, Seeley RJ.** Adiposity signals and the control of energy homeostasis. *Nutrition*. 2000;16:894–902.
  68. **Turri MG, Henderson ND, DeFries JC, Flint J.** Quantitative trait locus mapping in laboratory mice derived from a replicated selection experiment for open-field activity. *Genetics*. 2001;158:1217–26.
  69. **Feitosa MF, Borecki IB, Rich SS, et al.** Quantitative-trait loci influencing body-mass index reside on chromosomes 7

- and 13: the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Hum Genet.* 2002;70:72–82.
70. **Wu X, Cooper RS, Borecki I, et al.** A combined analysis of genomewide linkage scans for body mass index from the National Heart, Lung, and Blood Institute Family Blood Pressure Program. *Am J Hum Genet.* 2002;70:1247–56.
71. **Montague CT, Farooqi IS, Whitehead JP, et al.** Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature.* 1997;387:903–8.
72. **DiLeone RJ, Russell LB, Kingsley DM.** An extensive 3' regulatory region controls expression of Bmp5 in specific anatomical structures of the mouse embryo. *Genetics.* 1998;148:401–8.
73. **Lambertsson A.** The minute genes in *Drosophila* and their molecular functions. *Adv Genet.* 1998;38:69–134.
74. **Wainer H.** Adjusting for differential base rates: Lord's paradox again. *Psychol Bull.* 1991;109:147–51.
75. **Brockmann GA, Bevova MR.** Using mouse models to dissect the genetics of obesity. *Trends Genet.* 2002;18:367–76.
76. **Koza RA, Hohmann SM, Guerra C, Rossmeisl M, Kozak LP.** Synergistic gene interactions control the induction of the mitochondrial uncoupling protein (Ucp1) gene in white fat tissue. *J Biol Chem.* 2000;275:34486–92.
77. **Bachmanov AA, Reed DR, Beauchamp GK, Tordoff MG.** Food intake, water intake, and drinking spout side preference of 28 mouse strains. *Behav Genet.* 2002;32:435–43.
78. **Seburn KL.** Metabolic characterization. *Mouse Phenome Database.* 2001. <http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home> (accessed February 2003).
79. **Storer JB.** Relation of lifespan to brain weight, body weight, and metabolic rate among inbred mouse strains. *Exp Gerontol.* 1967;2:173–182.

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### Appendix 1. Phenotypic means of the Male A/J vs. C57BL/6J lines

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Trait	A/J mean (SD)	B6 mean (SD)	Reference
Ambulation in O-F (cm)	471 (215)	1402 (348)	(42)
Vertical rearings	19.3 (14.6)	93.0 (16.7)	(42)
AvgCtrT (sec)	4.5 (5.9)	29.4 (9.6)	(43)
L-D transitions	10.1 (9.0)	50.0 (9.8)	(43)
Tail suspension-induced hyperthermia (°C)	0.6	1.8	(34)
Daily average food intake adjusted for 30 g of body weight (g)	5.41	4.83	(77)
Caloric intake adjusted for 30 g of body weight (kcal/g)	7.91	9.82	(78)
Daily heat (kcal/kg/h) (females only)	0.404	0.399	(78)
Respiratory exchange ratio ( $V_{CO_2}:V_{O_2}$ ), daily (females only)	0.662	0.750	(78)
Mean metabolic rate ( $ccO_2/g/hr$ ) in 120 day old mice	4.08 (0.76)	4.55 (0.468)	(79)

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